REMARKS

Reconsideration of the instant application is respectfully requested in view of the previous amendments and the following remarks.

The status of claims 94-97, 101, 104-105, 109 and 111-113 has been changed to "Withdrawn." Claims 98, 100, and 102 have been amended. Amendments are supported at least by paragraph 0053 of the application as published ("[t]he formation of any complexes between the RlmA, or an rRNA binding domain thereof, and rRNA is then detected."

Claims 114-116 are added. The support for claim 114 is found at least in claim 98. The support for claim 115 is found in Fig. 1A (disclosing linear relationship of different β-sheets and α-helices), Fig. 1B disclosing spatial relationship between these structures, demonstrating that β-strands 3-11 and α-helices 1-8 and η1 form a methyltransferrase domain which binds rRNA.

Further support is found in paragraph 0026 of the application as published (US 20080057494), which discloses that beta strands β1- β3 form zinc binding domain which is different from the methyltransferrase domain, whose backbone is formed by β-strands 3-11.

The support for claim 116 is found at least in Fig. 1A.

The specification is amended according to the Examiner's recommendations. Support for the amendment reciting Figure 2C is found at least in paragraph 0139 of the application as published.

Accordingly, the amendments in this response do not add any new matter.

This invention is drawn, *inter alia*, to a method of identifying compounds having inhibitory activity against a bacterial strain, comprising preparing a cell-free reaction system comprising a bacterial RlmA protein or a rRNA binding domain thereof, a rRNA that binds said bacterial RlmA protein or the rRNA binding domain thereof, and a candidate compound and detecting the extent of binding between the bacterial RlmA protein or the rRNA binding domain thereof and the rRNA, wherein reduced binding between the bacterial RlmA protein or the rRNA binding domain thereof and the rRNA in the presence of the compound relative to a control is indicative of inhibitory activity of

the compound against the bacterial strain. This assay does not require S-adenosylmethionine or detection of S-adenosylhomocysteine.

A. Objection to Sequence Listing

Applicants submit an updated Sequence Listing along with Amendment to include this Sequence Listing into the instant disclosure.

B. Objection to drawings

The Examiner objected to drawings because, allegedly, Figure 2C was not mentioned in the specification. Applicants respectfully disagree and note that at least paragraph 0139 refers to FIG. 2C. Accordingly, the section "Brief Description of the Drawings" has been amended to include reference to FIG. 2C.

A new set of Drawings has also been submitted. The only changes between the instantly submitted Drawings and the previous Drawings is in FIG. 1A which provides SEQ ID NO identifiers to the sequences disclosed therein.

C. Objections to Specification

The Examiner objected to the specification due to typos in the abstract, lack of reference to the parent PCT application, and a hyperlink in the text. Amendments to the specification provided in the instant response comply with the Examiner's suggestions and thus overcome these grounds for objection. Accordingly, withdrawal of these objections is respectfully requested.

D. Rejection under 35 U.S.C. § 112, first paragraph (written description)

The Examiner rejected claims 98-100, 102-103, 106-108 and 110 as allegedly non-compliant with the written description requirement. Specifically, the Examiner asserts that Applicants were not in possession of the inhibitors of binding between rRNA and RlmA protein or an rRNA binding domain thereof. The Examiner further asserts that Applicants have not properly described rRNA binding domains of RlmA. Applicants respectfully challenge the Examiner's arguments.

As the Examiner correctly noted, that in order to comply with the written description requirement, Applicants have to show that they were in possession of the invention, i.e., "whatever is now claimed." Office Action at 7.

The claims are drawn to a method of identifying compounds affecting binding between rRNA and RlmA protein or an rRNA binding domain thereof. The compounds themselves are not claimed. Thus, the issue is whether a person of ordinary skill in the art would realize that applicants were in possession of a method for identification of such compounds.

Applicants provided a general description of suitable compounds as well as the source of the compounds. See at least paragraph 0073 of the application as published. The Examiner essentially states that the method of identifying compounds is not supported because the specific compounds suitable for being identified in this method are not described. Applicants respectfully note that the flaw in the Examiner's argument is that it is impossible to describe with particularity that which is to be identified.

Applicants respectfully repeat that the compounds themselves are not claimed. Accordingly, the cases cited by the Examiner, such as *Fiers v Revel* and *Amgen v Chugai* are inapplicable to this scenario since in those cases, the claims were drawn to the compounds (e.g., mammalian FGFs) rather than methods of identification of these compounds.

Applicants further respectfully refer the Examiner to Example 17 of the Written Description Training Materials ("Materials"). In that Example, claim 2 is drawn to a screening assay. Applicants specifically quote: "[T]he practice of the method [of identifying compounds having certain activity] requires no knowledge of the structures and properties of a compound that would predictably result in desired activity; rather the claimed invention is the screening process, not the compounds screened or compounds identified via claimed process." Materials, at 59. The analysis further concludes that the claim drawn to screening compounds for desired activity (akin to claim 98) complies with the written description requirement. Applicants respectfully request the Examiner to follow the Materials.

With regard to the argument that rRNA binding domains of RlmA has not been properly described, Applicants respectfully note that the sequences of RlmA genes of different bacterial strains are well known and publicly available, e.g., in BLAST or Genbank or Swissprot. Examples of the genes have been provided in the specification, e.g., Fig. 1A. The specification provides information on rRNA binding domain of E. coli

RlmA and compares E. coli RrmA with RrmA proteins from other bacterial strains. The specification further provides structural requirements for the functional rRNA binding domain. For example, paragraph 0142 states that

> The W-shaped putative rRNA-binding cleft (FIG. 2A) is comprised of conserved amino acid residues from both monomers of an asymmetric $RlmA^{I}$ dimer. Two Zn-fingers are at the top and the two SAM molecules are at the bottom of the cleft. At the bottom of the cleft, helices a1 from each monomer together form a ridge that separates the two SAM-binding pockets. The W-shaped cleft is lined with a positively charged electrostatic surface suitable for interactions with polyanionic nucleic acids (FIG. 4). The unusual asymmetric arrangement of $RlmA^{I}$ molecules in its dimer appears to be functionally relevant in creating the specific shape of the rRNA-binding cleft. The shape of the cleft is unique and different from that of previously reported RNA-binding proteins.

Paragraph 0143 discloses that rRNA binding cleft of $RlmA^{I}$, was clearly identifiable. Paragraph 0035 discloses that "[a]pplicants have discovered that $RlmA^{I}$ from E. coli dimerized in a specific fashion to define a 'W-shaped' binding cleft that would selectively recognize hairpin 35 of 23S RNA as its substrate." The applicants also predicted that the RNA-binding clefts of both $RlmA^{I}$ and $RlmA^{II}$ have similar folds and comparable shapes.

Paragraph 0119 discloses multiple software tools for analyzing the RlmA and discovering its binding domain (i.e., portions forming the W-shaped rRNA-binding cleft). Among these software tools are QUANTA, CHARMM; INSIGHT; SYBYL; MACROMODEL; and ICM.

Applicants further note that Fig. 1 and its description (at least paragraph 0026 of the application as published) disclose different β-sheets and α-helices present in RlmA of *E. coli*. The specification further discloses that the rRNA binding methyltransferrase domain of RlmA is composed of amino acids from strand β4 to the carboxy-terminus of that protein, while strands β1, β2 and β3 form a zinc-binding domain.

Figure 1A discloses sequence alignments between RlmA proteins from ten species. Notably, the specific structures (β-sheets and α-helices) are very well preserved among those species: the majority of amino acids among these sequences are either

identical or functionally equivalent (see, e.g., paragraph 0059 of the application as published). Accordingly, one of skill in the art would expect that the β-sheets and α-helices would be preserved among RlmA proteins of different species.

Applicants again respectfully refer the Examiner's attention to the Materials. Specifically, the technical note in Example 11 of the Materials ("Percent Identity") implies that approximately 50% sequence identity is likely to preserve the tertiary structure of a protein. On the next page, the Materials disclose that one of skill in the art would realize that "amino acids are grouped in so-called 'exchange groups' of similar properties because substituting within the exchange group is expected to conserve the overall structure."

Further, multiple examples in the Materials assume that a person of ordinary skill in the art uses software tools for determining whether the claims in those examples comply with the Written Description requirement. For example, in Example 12, the Materials assume that a person of ordinary skill in the art uses software tools for design of antisense nucleotides.

Similarly, in this case, in view of detailed disclosures in the specification and the high level of ordinary skill in molecular biology, one of ordinary skill in the art would be able to:

a) obtain the sequences for RlmA proteins of different bacterial species;

b) use computer modeling to model the structure of these proteins with reasonable certainty; and

c) identify the fragments of the molecules which form the rRNA binding W-shaped clefts.

Accordingly, for at least these reasons, Applicants respectfully submit that one of ordinary skill in the art would recognize that Applicants were in possession of rRNA binding domains of RlmA.

Therefore, for at least these reasons, Applicants respectfully request the Examiner to withdraw this rejection ground.

E. Rejection based on 35 U.S.C. § 112, second paragraph.

The Examiner rejected the claims as allegedly indefinite. Specifically, the Examiner argues that step (b) of claim 98 may be optional in the embodiments wherein only a rRNA binding domain of RlmA is used. Applicants thank the Examiner for noticing this typographical error. Claim 98, as well as claim 100, has been amended to recite the determination of binding of rRNA to the binding domain of RlmA. Accordingly, Applicants believe that these amendments overcome this rejection ground. In view of the above, withdrawal of this ground for rejection is respectfully requested.

F. Rejection based on 35 U.S.C. § 102(b)

The Examiner rejected claim 98 as allegedly anticipated by Gustafsson (*J. Bacteriol.* 180(2):359-365 (1998)). Applicants respectfully traverse.

As outlined in the summary paragraph of the introduction to Gustafsson et al:

> In this study, we localize the modified nucleotide on the rRNA, identify the gene (*rrmA*) encoding the rRNA-modifying enzyme, and analyze the growth characteristics and susceptibility to antibiotics of an *rrmA* mutant

Gustafsson does not work with or describe the use of a RlmA protein, as required by claim 98. Gustaffson also does not disclose the step of measuring the extent of complex formation between rRNA and the RlmA, as required by claim 98. These differences are sufficient to conclude that claim 98 is not anticipated by Gustafsson, since a reference anticipates a claim only it discloses each and every limitation of the claim.

Nevertheless, solely for expediting the prosecution of the instant application, claim 98 has been amended to recite a cell-free reaction system, which is neither disclosed nor suggested in Gustaffson.

Accordingly, withdrawal of this rejection ground is respectfully requested.

G. Rejection based on 35 U.S.C. § 102(e) and 103

The Examiner rejected the claims of the instant application as allegedly anticipated under § 102(e) in view of Yuan (US 6,610,504). The Examiner also rejected the claims of the instant application as allegedly obvious over Yuan and Liu (*PNAS* 99(23): 14658-14663 (2002)). Applicants respectfully traverse and, for the purpose of efficiency, address both rejections together.

Methylation of rRNA by RlmA involves transferring a methyl group from S-adenosylmethionine to rRNA. In this process, S-adenosylmethionine, the donor of the methyl group, is converted to S-adenosylhomocysteine. Yuan's method involves detection of S-adenosylhomocysteine in a sample, thus accessing the rate of methyl transfer. It is based on measurement of a ***functional*** activity. Yuan does not disclose or suggest measurement of the binding between RlmA and rRNA. In contrast, the instant invention detects the ***physical binding*** between the rRNA and RlmA or the rRNA binding domain thereof. Thus, Yuan does not anticipate the claims of the invention of claim 98.

Applicants further respectfully note that formation of S-adenosylhomocysteine cannot be considered a reliable indirect measurement of the formation of a complex between rRNA and RlmA or rRNA binding domain thereof. Briefly, the conversion of S-adenosylmethionine to S-adenosylhomocysteine is a multistep process that includes binding of RlmA to substrate rRNA, binding of S-adenosylmethionine to RlmA, catalysis of the methyl transfer and formation of S-adenosylhomocysteine, and release of S-adenosylhomocysteine. Each of these steps is characterized by different reaction kinetics, with different equilibrium and rate constants. These constants are independent of each other. The formation of S-adenosylhomocysteine is a product of interaction of all four reactions. Thus, for example, the binding of RlmA to rRNA may be very weak but rapid catalysis could result in extensive production of S-adenosylhomocysteine with minimal binding. On the other hand, tight binding of RlmA to rRNA and slow catalysis would result in little or no S-adenosylhomocysteine.

In view of the above, in a scenario where a test compound significantly decreases the formation of S-adenosylhomocysteine, it is unknown (and unknowable, using Yuan's method) which reaction (rRNA binding, S-adenosylmethionine binding, methyl transfer, or release of S-adenosylhomocysteine) is affected by the test compound. Accordingly, the detection of S-adenosylhomocysteine is not a measurement of the ***extent of binding*** between Rlm and rRNA.

The other claims are related to different embodiments of claim 98, and some of those claims recite features which are neither disclosed not suggested in Yuan. For example, claim 102 (and claims dependent therefrom) specify that rRNA or RlmA (or its

rRNA binding domain) are labeled with certain labels including the elected fluorescent label. The Examiner asserts that Yuan discloses the use of fluorescent labels, but this is a simplistic analysis, which disregards the nature of the compound which is being labeled. Yuan discloses labeling S-adenosylhomocysteine (or its precursor S-adenosylmethionine) or the enzyme which binds S-adenosylhomocysteine (so-called mutant enzyme). Labeling of the compounds recited in claim 102, namely rRNA, RlmA, or rRNA binding domain of RlmA is neither disclosed nor suggested. Therefore, for at least these reasons, Applicants respectfully disagree that claim 102 is anticipated.

Applicants further note that Yuan does not disclose or suggest preparing a reaction system does not require S-adenosylmethionine. In fact, Yuan's invention would be inoperable if his reaction system did not include S-adenosylmethionine. In other words, if S- adenosylmethionine is not added, then S-adenosylhomocysteine is not formed. If S-adenosylhomocysteine is not formed, there is nothing to detect, using the method of Yuan.

Accordingly, some other parameter has to be detected if S-adenosylmethionine is absent in the system. Measuring this "other" parameter would lead to material changes in the principle of operation of Yuan's method.

Further, Liu does not cure the deficiencies of Yuan discussed above. Liu only discloses screening of compounds affecting bacterial growth in cell-based assays. In Liu's system, RlmA is provided only as a part of a cell. Thus, Liu's system is not cell-free and does not lack S-adenosylmethionine. Further, Liu does not disclose or suggest measuring the extent of binding between rRNA and RlmA or rRNA binding domain thereof.

According to MPEP § 2143.01.V and MPEP § 2143.01.VI, proposed modifications may not render prior art inoperable for its intended purpose and proposed modifications may not change the principle of operation of a reference.

Accordingly, for at least these reasons, the claims of the instant invention are not obvious in view of the combination of Yuan and Liu.

Further, the inventors have proposed that in the transfer of a methyl group catalyzed by RlmA, RlmA needs ***both*** S-adenosylmethione and rRNA in order to convert S-adenosylmethionine to S-adenosylhomocysteine (see paragraph 0139, stating that "the

RNA substrate is necessary for a SAM molecule to bind to the $RlmA^I$ enzyme in a proper orientation for MTase catalysis."). Thus, Yuan's system requires both rRNA ***and*** S-adenosylmethionine. In contrast, in the instant invention does not need a methyl group donor. Accordingly, the instant invention achieves its purposes with fewer steps and fewer ingredients, as compared to Yuan's assay. Thus, the instant invention achieves its function without elements (S-adenosylmethionine) or steps (detection of S-adenosylhomocysteine) which are crucial in Yuan. This simplified and more economical design of the instant invention is another evidence of non-obviousness of claim 98 and claims dependent therefrom in view of Yuan and Liu.

Therefore, for the reasons above, Applicants respectfully request withdrawal of this ground for rejection.

CONCLUSION

Applicants respectfully submit that the pending claims are valid and favorable reconsideration and allowance are earnestly solicited. If, however, for any reason the Examiner does not believe that such action can be taken at this time, it is respectfully requested that the Examiner telephone Applicant's attorney at (609) 844-3020 to discuss any additional rejections.

The USPTO is authorized to charge Deposit Account No. 50-1943 for any fees due.

Dated: June 26, 2009

Respectfully submitted,

By: /Gerard P. Norton/

Gerard P. Norton, Reg. No. 36,621
Fox Rothschild LLP
997 Lenox Drive, Building 3
Princeton, New Jersey 08648
Telephone: (609) 844-3020
Facsimile: (609) 896-1469
Attorneys for Applicant(s)